Bayer

Preliminary Clinical Method
Premarket Approval

Publication No. DA4-1210XXX

July 2000

Bayer Immuno 1™ System

COMPLEXED PROSTATE SPECIFIC ANTIGEN (cPSA)

TECH-CHECK™ Table

Method Principle	Heterogeneous Sandwich Magnetic Separation Assay (MSA)
Analytical Range	0.02 ng/mL to approximately 100.0 ng/mL
Specimen Type	Human serum
Sample Test Volume	20.0 µL
Minimum Fill	Refer to "SAMPLE COLLECTION AND PREPARATION" in the INTRODUCTION to *Bayer Immuno 1 Methods Manual.*
Sensitivity	0.02 ng/mL
Reference Material	Stanford University PSA Reference Material
Common Units	ng/mL
Upper Limit of Normal	**3.6 ng/ml**

INTENDED USE

This *in vitro* diagnostic assay is intended to quantitatively measure complexed prostate specific antigen (cPSA) in human serum on the *Bayer Immuno 1* system. This device is indicated for the measurement of serum complexed PSA in conjunction with a digital rectal exam (DRE) as an aid in the detection of prostate cancer in men aged 50 years or older. Biopsy of the prostate is required for the diagnosis of prostate cancer. This device is further indicated as an aid in management (monitoring) of prostate cancer patients.

This diagnostic method is not intended for use on any other system.

WARNING! The concentration of complexed Prostate Specific Antigen (cPSA) in a given specimen will differ from concentrations determined using cPSA or PSA assays from other manufacturers. The results reported by the laboratory to the physician must include the identity of the cPSA or PSA assay. Values obtained with assays that measure total PSA cannot be used interchangeably with cPSA assay values. If, in the course of monitoring the patient, the assay used for determining PSA or cPSA levels is changed, additional sequential testing should be carried out to confirm baseline values.

Federal law restricts this device to sale and distribution by or on the order of a physician, or to a clinical laboratory; and use is restricted to, by, or on the order of a physician.

SUMMARY AND EXPLANATION

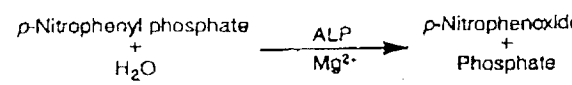
Prostate specific antigen is a neutral serine protease composed of a single polypeptide chain of 237 amino acids and having a mature molecular weight of approximately 33,000 Daltons.[1] Studies from several laboratories have established that measurement of serum PSA has value in monitoring patients with prostate cancer and in the early detection of localized and, therefore, potentially curable prostate cancer.[2-4] Despite the widespread acceptance and use of serum PSA for early detection of prostate cancer, the specificity of PSA testing is relatively low.[5-7]

Several approaches have been suggested to improve the specificity of PSA testing. These include the use of serum PSA and prostate gland volume to develop a ratio termed PSA density,[8] longitudinal measurement of serum PSA values to develop the rate of increase in PSA per year, or PSA velocity,[9] and the application of age-adjusted reference ranges to compensate for the known increase in prostate gland volume in men over the age of 50 years.[10, 11] Despite promising evidence in the clinical literature, none of these approaches has gained widespread acceptance. Another approach to improve PSA specificity stems from the observation that the majority of immunoreactive PSA in cancer patients is in complex with alpha-1-antichymotrypsin (ACT).[12-14]

The Bayer Immuno 1 system cPSA assay accurately measures PSA in complex with protease inhibitors. This assay has value in the discrimination of prostate cancer from benign prostate disease, and in the monitoring of prostate cancer following primary treatment.

PRINCIPLES OF THE PROCEDURE

This method uses a sandwich immunoassay format. Complexed PSA (cPSA) Antibody Conjugate 1 (R1) and Complexed PSA (cPSA) Antibody Conjugate 2 (R2) are reacted with patient sample [or calibrator containing Complexed PSA (cPSA)] and incubated on the system at 37 °C. The *mIMP* ™ (monoclonal Immuno Magnetic Particle) Reagent is added and a second incubation occurs during which the antibody complex is bound. The mIMP/antibody complex is then washed and the *p*NPP (para-nitrophenyl phosphate) substrate is added. The alkaline phosphatase (ALP) in the antibody conjugate reacts with the *p*NPP to form para-nitrophenoxide and phosphate. Increasing absorbance, due to the formation of para-nitrophenoxide, is monitored at 405 nm and 450 nm. The indicator reaction occurs as follows:



A sample having no cPSA will have the minimum label bound, while samples containing high cPSA concentrations will have the maximum label bound. Thus, the dose response curve is directly proportional to the cPSA concentration in the sample.

24

REAGENTS

Material Provided

The following materials are available and provided in the package sizes listed in Tables 1a, 1b, and 1c. Components of the packages are sold as a kit and not sold separately.

Table 1a: REAGENT PACKAGING INFORMATION

PROD. NO.	CONTAINS	FILL VOLUME (mL)	NUMBER OF TESTS
T01-3982-51	Complexed PSA (cPSA) Reagents	2 x 9.0	100

NOTE: Materials in these reagents are light sensitive. Once removed from the carton, the reagent must either be placed on the system as soon as possible or kept in a dark, refrigerated area to avoid exposure to light.

"For *In Vitro* Diagnostic Use."

The Packaging of This Product Contains Dry Natural Rubber.

Each carton contains:

Complexed PSA (cPSA) Antibody Conjugate (R1)
(Printed Label Side)
As formulated contains: Mouse monoclonal anti-PSA, 1.54 mg/L (nominal quantity); Buffer; Surfactant; 0.095% Sodium azide; Preservative

NOTE: Handle as any patient sample. Avoid contact with eyes, skin, or clothing. Wash thoroughly after handling. Avoid ingestion.

Complexed PSA (cPSA) Antibody Conjugate (R2)
(Barcode Label Side)
As formulated contains: Goat polyclonal anti-PSA ALP conjugate, 6.15 mg/L (nominal quantity); Mouse monoclonal unlabeled PSA antibody, 100 mg/L; Buffer; Surfactant; 0.095% Sodium azide; Preservative

NOTE: Handle as any patient sample. Avoid contact with eyes, skin, or clothing. Wash thoroughly after handling. Avoid ingestion.

CAUTION! Contains sodium azide. After contact with skin, wash immediately with plenty of water. Because sodium azide may form lead or copper azides in plumbing, it is recommended that drains be thoroughly flushed with water after disposal of solutions containing sodium azide. See Technical Bulletin TT6-0319-11.

CALIBRATORS

Table 1b: CALIBRATOR PACKAGING

PROD. NO.	CONTAINS	FILL VOLUME (mL)
T03-3983-01	*Bayer®SETpoint™* Complexed PSA (cPSA) Calibrators	1 x 5.0 5 x 1.0

NOTE: Do not intermix components of different lots of Bayer SETpoint Complexed PSA (cPSA) Calibrators.

Bayer is a registered trademark of Bayer AG.

Each carton contains:

***Bayer SETpoint* Complexed PSA (cPSA) Calibrator 1**
(Prod. No. T23-3983-01) 1 x 5.0 mL
Each vial contains: Bovine serum albumin, 0.095% Sodium azide

***Bayer SETpoint* Complexed PSA (cPSA) Calibrator 2**
(Prod. No. T23-3983-02) 1 x 1.0 mL
Each vial contains: PSA-ACT, Bovine serum albumin, 0.095% Sodium azide

***Bayer SETpoint* Complexed PSA (cPSA) Calibrator 3**
(Prod. No. T23-3983-03) 1 x 1.0 mL
Each vial contains: PSA-ACT, Bovine serum albumin, 0.095% Sodium azide

***Bayer SETpoint* Complexed PSA (cPSA) Calibrator 4**
(Prod. No. T23-3983-04) 1 x 1.0 mL
Each vial contains: PSA-ACT, Bovine serum albumin, 0.095% Sodium azide

***Bayer SETpoint* Complexed PSA (cPSA) Calibrator 5**
(Prod. No. T23-3983-05) 1 x 1.0 mL
Each vial contains: PSA-ACT, Bovine serum albumin, 0.095% Sodium azide

***Bayer SETpoint* Complexed PSA (cPSA) Calibrator 6**
(Prod. No. T23-3983-06) 1 x 1.0 mL
Each vial contains: PSA-ACT, Bovine serum albumin, 0.095% Sodium azide

CONTROLS

Table 1c: CONTROL PACKAGING

PROD. NO.	CONTAINS	FILL VOLUME (mL)
T03-3984-01	*Bayer®SETpoint™* Complexed PSA (cPSA) Controls	6 x 2.0

NOTE: Do not intermix components of different lots of Bayer TESTpoint Complexed PSA (cPSA) Controls.

***Bayer TESTpoint* Complexed PSA (cPSA) Control 1**
(Prod. No. T23-3984-01) 2 x 2.0 mL
Each vial contains: PSA-ACT, Bovine serum albumin, 0.095% Sodium azide

***Bayer TESTpoint* Complexed PSA (cPSA) Control 2**
(Prod. No. T23-3984-02) 2 x 2.0 mL
Each vial contains: PSA-ACT, Bovine serum albumin, 0.095% Sodium azide

Bayer TESTpoint **Complexed PSA (cPSA) Control 3**
(Prod. No. T23-3984-03) 2 x 2.0 mL
Each vial contains: PSA-ACT, Bovine serum albumin, 0.095% Sodium azide

***WARNING!* - POTENTIALLY BIOHAZARDOUS MATERIAL**

Human sourced materials were used in the manufacturing of this product. Each donor unit was tested for hepatitis B surface antigen (HBsAg), antibodies to hepatitis C (HCV), and antibodies to Human Immunodeficiency Viruses (HIV-1 and HIV-2) and found to be negative (was not repeatedly reactive). If no donor blood sample was available, an extract of the starting material was tested and found negative (was not repeatedly reactive) for hepatitis B surface antigen (HBsAg), antibodies to hepatitis C (HCV), and antibodies to Human Immunodeficiency Viruses (HIV-1 and HIV-2).

CAUTION: Because no test method can offer complete assurance that HIV, hepatitis B or C viruses, or other infectious agents are absent, these products should be handled at the Biosafety Level II as recommended for any potentially infectious human blood specimens in *Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue*, 2d edition, Approved Guideline (1997), Document M29-A, promulgated by the National Committee for Clinical Laboratory Standards (NCCLS).

WARNING! Contains sodium azide. Harmful if swallowed. After contact with skin, wash immediately with plenty of water. Because sodium azide may form lead or copper azides in plumbing, it is recommended that drains be thoroughly flushed with water after disposal of solutions containing sodium azide. See Technical Bulletin TT6-0319-11.

NOTE:
Other system solutions and controls are necessary to perform this method. Refer to the listing of these solutions and controls, along with the instructions for their preparation and use, in the INTRODUCTION to the *Bayer Immuno 1 System Methods Manual.*

Reagent, Calibrator, and Control Preparation

Complexed PSA (cPSA) Reagents (R1 and R2) are supplied in a ready to use liquid form, packaged in the reagent cassette. When handling a cassette, do not fill the base portion by tipping it. The system automatically fills the base of the cassette to the proper level. Do not spill reagent on the evaporation covers and be careful to prevent cross-contamination of the R1 and R2. Discard shipping stoppers. Do not reseal the cassette by using old stoppers or by pressing down on the evaporation covers.

If for some reason the evaporation cover must be manually opened, do not force the cover through its full range of travel. This could overcompress and damage the spring in the cover. Open the cover only to the point at which a clear path through the aspiration port can be seen.

If cassettes are to be removed from the system and temporarily stored in a 2 °C to 8 °C refrigerator, **protect the contents from exposure to light**. Evaporation covers provide adequate dust and evaporation protection for refrigerator storage.

Each of the Bayer SETpoint Complexed PSA (cPSA) Calibrators and Bayer TESTpoint Complexed PSA (cPSA) Controls is frozen and must be prepared according to the following instructions:

1. Thaw at 2 °C to 8 °C prior to initial use.
2. Break vial closure.
3. Swirl gently, then mix by inversion at least five (5) times to ensure homogeneity prior to use.
4. Refrigerate any unused material. Prior to reuse, mix contents thoroughly.

STORAGE AND STABILITY

Unopened reagents, when stored at 2 °C to 8 °C, and unopened calibrators or controls, when frozen at ≤ -10 °C in a freezer that is not frost-free, are stable through the last day of the month (expiration date) printed on the product label.

After being opened, calibrators and controls are stable **at least thirty-five (35) days** when stored stoppered in their original containers at a temperature of 2 °C to 8 °C and kept free of contamination. The reagents have been tested over a wide range of laboratory conditions and found stable on system for **at least twenty-one (21) days.**

SAMPLE HANDLING[23]

Serum samples should be tested within twenty-four hours after collection when stored at 2 °C to 8 °C. If testing is to be delayed for more than twenty-four hours, store the samples frozen. Frozen samples should be thawed, mixed thoroughly, and centrifuged before use to ensure consistency of results. Avoid repeated freezing and thawing.

MATERIALS REQUIRED BUT NOT PROVIDED

The materials required that are not provided to perform this method are Bayer Immuno 1 system, reaction tray, *Technicon IDee®* labels, sample cups, control materials, Class A or equivalent volumetric pipette, and other reagents and equipment as specified in the Introduction to the *Bayer Immuno 1 System Methods Manual.*

PROCEDURE

Entering Chemistry and Calibration Program

The chemistry parameters for this method are resident on the system. At initial installation, this assay and its specific parameters must be updated from the library disk. The cPSA concentrations for the calibrators must be entered on the system to two (2) decimal places. The cPSA calibrator concentrations are lot specific and can be found in the insert packaged with the calibrators.

After the reagent cassette is placed into the reagent tray and the door is closed, an automatic reagent scan, which enters the reagent information into the REAGENT INVENTORY, is performed. A partially used reagent cassette *must not* be switched to another Bayer Immuno 1 system, as this will result in an incorrect inventory.

Refer to the OPERATION section of the *Bayer Immuno 1 System Operator's Manual* for further information.

QUALITY CONTROL

It is recommended that the system be controlled using Bayer TESTpoint Complexed PSA (cPSA) Controls (Prod. No. T03-3984-01). These controls are intended to be integrated into a clinical laboratory's own quality control program and procedures.

These controls should be assayed:

1. At the beginning of each shift or at some other interval chosen by the laboratory.

2. Whenever a reagent cassette is depleted and another of the same lot is installed, prior to reporting patient results.
3. Whenever a new lot of reagent is used.
4. Following maintenance or cleaning of any detection system components.

A satisfactory level of performance is achieved when the analyte values obtained for each control are within the "Acceptable Control Range" published in the Package Insert provided with the control material or in a subsequent Product Notification containing value reassignment information.

CALIBRATION

Calibration of this method is performed with Bayer SETpoint Complexed PSA (cPSA) Calibrators (Prod. No. T03-3983-01), which contain six individual calibrator levels. This method utilizes a cubic algorithm for developing the calibration curve. The calibration curve must be reviewed and accepted using the CALIBRATION REVIEW SCREEN. The curve can be printed from the CALIBRATION REVIEW SCREEN.

A set of values defining the acceptable limits for the fitting of the calibrators ensures that unsatisfactory data are not used. In the subsection entitled "Calibration Review," in the CALIBRATION section of the *Bayer Immuno 1 System Operation Manual,* there are detailed explanations of possible error conditions and their related corrective actions.

Calibration Schedule

Calibration should be performed when this method is implemented on the Bayer Immuno 1 system.

Recalibration is required after replacement of major components; a change in the lot number for Complexed PSA (cPSA) reagents, mIMP Reagents, or Substrate Reagents; or as indicated by quality control results.

Based on our findings, the minimum calibration stability for this method is thirty (30) days. This is based on control results remaining within ±2 total standard deviations or ±10% (whichever is greater) of the control mean value concentration for the assay.

Calibration Procedure

Instructions for calibrating an immunoassay method are provided in the subsection titled "Calibration Procedure," in the CALIBRATION section of the *Bayer Immuno 1 System Operation Manual.*

Reference Material[24]

This method is traceable to the Stanford University PSA Reference Material.

RESULTS

Complexed PSA (cPSA) patient sample results that are greater than the highest fitted value on the immunoassay "CALIBRATION" report will have the actual results replaced with a ">" flag on the Bayer Immuno 1 system patient report.

Dilute any over-range samples with Bayer 1 SETpoint Complexed PSA Calibrator 1 (Prod. No. T23-3983-01) or Bayer Immuno 1 Sample Diluent B (Prod. No. T03-3574-01), using a volumetric pipette, Class A or equivalent, to bring the concentration within the calibration curve, then reassay the diluted sample.

No high-dose hook effect will be apparent for cPSA concentrations up to 12,500 ng/mL.

In certain cases, as outlined in the subsection titled "Viewing the Sample Log," which appears in the DURING RUN section of the *Bayer Immuno 1 System Operation Manual,* results may be replaced with a flag when error-checking algorithms are exceeded

LIMITATIONS OF THE PROCEDURE[23, 25-31]

As with any immunochemical reaction, users should be alert to the possible effect on test results of potential interference from medications or unknown endogenous substances. All patient results should be evaluated in light of the total clinical status of the patient. Refer to the paragraph entitled, "Interpretation of Results," contained in the INTRODUCTION to the *Bayer Immuno 1 System Reference Manual, UNIT 4.*

Samples from patients receiving preparations of mouse monoclonal antibodies for therapy or diagnosis may contain human anti-mouse antibodies (HAMA). Such samples may show either falsely elevated or falsely depressed values when tested with this method and should not be assayed.

Patient samples containing significant levels of rheumatoid factor (RF) or heterophilic antibodies may produce falsely elevated or falsely depressed values when tested with this assay. Potential interference caused by the presence of these substances should be considered when interpreting assay results that are inconsistent with the total clinical status of the patient.

Evidence suggests that patients undergoing retinal fluorescein angiography may retain amounts of fluorescein in the body for up to 36 to 48 hours post-treatment. In the cases of patients with renal insufficiency, including many diabetics, retention may be much longer. Such samples may show either falsely elevated or falsely depressed values when tested with this method and should not be assayed.

It is possible that a patient with confirmed prostatic cancer may have serum Complexed PSA (cPSA) levels within the range of those observed in healthy individuals. Elevated Complexed PSA (cPSA) levels also can be found in patients with nonmalignant diseases of the prostate along with other adjacent genitourinary tissues. Elevated serum cPSA concentrations can only suggest the presence of prostate cancer; performance of biopsy is required for diagnosis.

Hormonal therapy can affect Complexed PSA (cPSA) expression; therefore, low Complexed PSA (cPSA) measurement after this type of treatment may not adequately reflect the presence of residual or recurrent disease.

Do not interpret Complexed PSA (cPSA) results as absolute evidence of the presence or absence of malignant disease. The Complexed PSA (cPSA) value should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

EXPECTED VALUES

Healthy Men

As with all tests, each laboratory should establish its own reference range. Since Complexed PSA is a proportion of serum total PSA, the upper limit of normal can be expected to be different than that for the total PSA. It is normally expected that about 90% of the serum PSA is complexed. The *Bayer Immuno 1* Complexed PSA (cPSA) assay (Prod. No. T01-3982-51) with an upper limit of normal of 3.60 ng/mL gave equivalent sensitivity to the *Bayer Immuno 1* PSA assay (Prod.

COMPLEXED PROSTATE SPECIFIC ANTIGEN (cPSA)

METHOD No. DA4-1210XXX

No. T01-3450-51) using an upper limit of normal of 4.0 ng/mL. The distribution for Complexed PSA (cPSA) values in a study of 280 apparently healthy males of ages ranging from 40 to 82 is shown in Figure 1. Some studies indicate that PSA values tend to rise with age.[11]

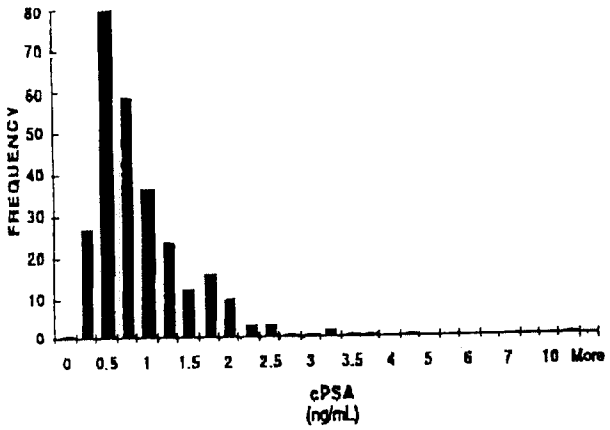


Figure 1: DISTRIBUTION OF COMPLEXED PSA (cPSA) RESULTS IN APPARENTLY HEALTHY MALES (40 to 82 YEARS OLD)

Detection Of Prostate Cancer

A multicenter clinical trial was conducted to test the effectiveness of cPSA along with DRE as an aid in the detection of prostate cancer. A total of 3,268 men aged 50 or older participated in the study. In a population of 356 biopsied men, 125 men or 35.1% were found to have prostate cancer. This study also demonstrated that cPSA testing, when used in conjunction with DRE was more effective in detecting cancer than DRE alone. The added value of cPSA determinations in combination with DRE detected 30.4% (38/125) of cancers that DRE alone did not.

cPSA elevations greater than 3.6 ng/mL may warrant additional testing even if the DRE is negative. However, the converse is also true; a subject with suspicious DRE and a normal cPSA may also require additional testing, since DRE detected 15.2% (19/125) of cancers that cPSA determinations did not.

Based on biopsy and DRE, 84% of cancers were of early stage (T1 or T2) and 84% were of low grade (Gleason scores of ≤ 7). A summary of the study results is provided in Table 2.

Table 2: CLINICAL TRIAL RESULTS

	NUMBER OF SUBJECTS (%)	NUMBER OF BIOPSIES	NUMBER OF NON-CANCERS	NUMBER OF CANCERS	% POSITIVE BIOPSIES
All Subjects	3,268 (100%)	356	231	125	35
DRE +	383 (11.72%)	146	81	65	45
DRE -	2,885 (88.28%)	210	150	60	29
cPSA > 3.6	520 (15.91%)	266	183	103	39
cPSA ≤ 3.6	2,748 (84.09%)	90	68	22	24
cPSA ≤ 3.6 and DRE -	2,487 (76.10%)	19	16	3	16
cPSA > 3.6 and DRE -	398 (12.18%)	191	134	57	30
cPSA ≤ 3.6 and DRE +	261 (7.99%)	71	52	19	27
cPSA > 3.6 and DRE +	122 (3.73%)	75	29	46	61
tPSA > 4.0 ng/mL	592 (18.1%)	282	175	107	38
tPSA ≤ 4.0 ng/mL	2676 (81.9%)	74	56	18	24
tPSA ≤ 4.0 and DRE -	2435 (74.5%)	13	11	2	15
tPSA > 4.0 and DRE -	450 (13.8%)	197	139	58	29
tPSA ≤ 4.0 and DRE +	241 (7.4%)	61	45	16	26
tPSA > 4.0 and DRE +	142 (4.3%)	85	36	49	58

DRE+ Suspicious for cancer
DRE– Not suspicious for cancer

Longitudinal Monitoring

In a separate multicenter clinical trial with 155 men previously treated for prostate cancer, longitudinal measurement of serum concentrations of complexed PSA (cPSA) was found to mirror changes in disease status for 150 (97%) of these patients. Complexed PSA (cPSA) assay values were also shown to correlate with trends obtained using the Bayer Immuno 1 PSA assay.

Two examples of longitudinal studies with *Bayer Immuno 1* Complexed PSA (cPSA) assay results when compared with the Bayer Immuno 1 PSA assay (Prod. No. T01-3450-51) are shown in Figures 3 and 4.

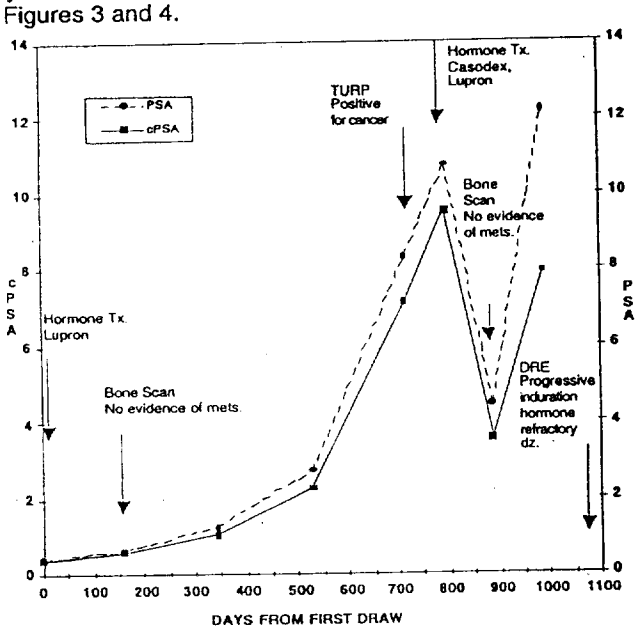


Figure 3: COMPLEXED PSA (cPSA) SERIAL MONITORING CLINICAL STATUS INCLUDES DISEASE PROGRESSION AND RESPONSE TO THERAPY

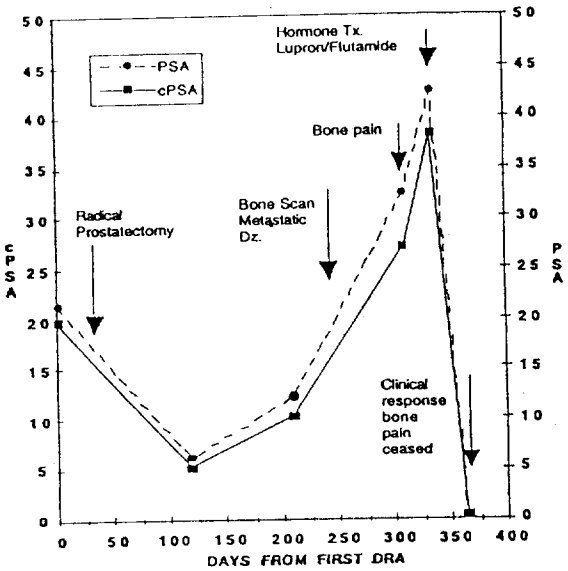


Figure 4: COMPLEXED PSA (cPSA) SERIAL MONITORING CLINICAL STATUS INCLUDES DISEASE PROGRESSION AND RESPONSE TO THERAPY

PERFORMANCE CHARACTERISTICS

Imprecision

The estimates of imprecision shown in Table 3 were obtained from replicate assays of human serum pools and controls. Imprecision estimates were collected in twenty (20) runs over ten (10) days at three (3) sites and computed according to NCCLS document EP5-A*, *Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline.*

Table 3: IMPRECISION

SOURCE	LEVEL (ng/mL)	TOTAL SD (ng/mL)	TOTAL CV (%)	WITHIN-RUN SD (ng/mL)	WITHIN-RUN CV (%)
Serum Pool	0.67	0.02	2.3	0.01	1.3
Control 1	3.35	0.08	2.3	0.06	1.9
Control 2	14.88	0.30	2.0	0.24	1.6
Control 3	74.34	1.76	2.4	1.38	1.9

SENSITIVITY

Minimum Detectable Concentration

The minimum detectable concentration of Complexed PSA (cPSA) is 0.02 ng/mL. This is a multisystem estimate of two (2) times the within-run standard deviation of the zero calibrator.

SPECIFICITY

Interfering Substances

The use of hemolyzed (up to 1.0 g/dL of hemoglobin), lipemic (up to 3000 mg/dL of triglycerides), icteric (up to 25 mg/dL of total bilirubin), albumin (up to 6.5 g/dL), immunoglobulin (up to 6.0 g/dL of IgG), and heparin (up to 0.46 mg/dL) samples has no clinically significant effect on method performance.

Cross-Reactivity

Cross-reactivity has been tested with several compounds that could interfere in the assay. A compound is considered cross-

reactive if its presence provokes a 10% error in the value of a Complexed PSA (cPSA) sample. None of the compounds tested showed cross-reactivity at the levels indicated in Table 4.

Table 4: CROSS-REACTIVITY

CROSS-REACTANT	CROSS-REACTANT CONCENTRATION
Aminoglutethamide	398 µg/mL
Bleomycin	0.16 U/mL
Cis-Platin	173 µg/mL
Cyclophosphamide	800 µg/mL
Diethylstilbestrol	23 µg/mL
Doxorubicin	51.8 µg/mL
Esramustine	102.2 µg/mL
Flutamide	10 µg/mL
5-Fluorouracil	1600 µg/mL
Lupron	15 µg/mL
Methotrexate	450 µg/mL
Mixoxantrone	56 µg/mL
Mitomycin	73 µg/mL

* Available from National Committee for Clinical Laboratory Standards, 940 West Valley Road, Suite 1400, Wayne, PA 19085-1898

Analytical Range

The analytical range for this method extends from the minimum detectable concentration of Complexed PSA (cPSA) [0.02 ng/mL] to the concentration of Complexed PSA (cPSA) in Calibrator Level 6. Samples with a concentration greater than the upper limit of the analytical range should be diluted using a volumetric pipette, Class A or equivalent, with Bayer SETpoint Complexed PSA (cPSA) Calibrator Level 1 or Bayer Immuno 1 Sample Diluent-B (Prod. No. T03-3574-01), and reassayed.

BIBLIOGRAPHY

1 Watt KWK, *et al.* Human prostate-specific antigen: Structural and functional similarity with serine proteases. *Proc Natl Acad Sci* **83**:3166-3170 (1986)

2 Stamey TA, *et al.* Prostate-specific antigen as a serum marker for adenocarcinoma of the prostate. *N Eng J Med* **317**:909-916 (1987)

3 Catalona WJ, *et al.* Measurement of prostate-specific antigen in serum as a screening test for prostate cancer. *N Eng J Med* **324**:1156-1161 (1991)

4 Partin AW and Oesterling JE: The clinical usefulness of prostate specific antigen: Update 1994. *J Urol* **152**:1358-1368 (1994)

5 Catalona WJ, *et al.* Comparison of digital rectal examination and serum prostate specific antigen in the early detection of prostate cancer: Results of a multicenter trial of 6630 men. *J Urol* **151**:1283-1290 (1994)

6 Brawer MK, *et al.* Screening for prostate carcinoma with prostate specific antigen. *J Urol* **147**:841-845 (1992)

7 Ellis WJ, Chetner MP, Preston SD, and Brawer MK: Diagnosis of prostatic carcinoma: The yield of serum prostate specific antigen, digital rectal examination and transrectal ultrasonography. *J Urol* **152**:1520-1525 (1994)

8 Benson MC, *et al.* Prostate specific antigen density: A means of distinguishing prostatic hypertrophy and prostate cancer. *J Urol* **147**:815-816 (1992)

9 Carter HB, *et al.* Longitudinal evaluation of prostate-specific antigen levels in men with and without prostate disease. *JAMA* **267**:2215-2220 (1992)

10 Oesterling JE, *et al.* Free, complexed and total serum prostate specific antigen: The establishment of appropriate reference ranges for their concentrations and ratios. *J Urol* **154**:1090-1095 (1995)

11 Oesterling JE, *et al.* Serum prostate-specific antigen in a community-based population of healthy men. Establishment of age specific reference ranges. *JAMA* **270**:860 (1993)

12 Stenman U-H, *et al.* A complex between prostate-specific antigen and α-1-antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostatic cancer: Assay of the complex improves clinical sensitivity for cancer. *Can Res* **51**:222-226 (1991)

13 Lilja H, *et al.* Prostate-specific antigen in serum occurs predominantly in complex with α-1-antichymotrypsin. *Clin Chem* **37**:1618-1625 (1991)

14 Lilja H, Cockett ATK, and Abrahamsson P-A: Prostate specific antigen predominantly forms a complex with α-1-antichymotrypsin in blood. *Can* **70**:230-234 (1992)

15 Pettersson K, *et al.* Free and complexed prostate-specific antigen: In vitro stability, epitope map, development of immunofluorometric assays for specific and sensitive detection of free PSA and PSA-α-1-antichymotrypsin complex. *Clin Chem* **41**:1480-1488 (1995)

16 Chan DW, Kelley CA, and Partin AW: PSA-ACT immunoassay: Problems and solutions. *Clin Chem* **42**:S255 (1996)

17 Zhang P and Wu JT: Development of an immunoassay specific for the PSA-ACT complex in serum without interference of non-specific adsorption. *Clin Chem* **43**:S236 (1997)

18 Chan DW and Sokoll LJ: Prostate specific antigen: Update 1997. *J Int Fed Clin Chem* **9**:120-125 (1997)

19 Wang TJ, *et al.* Development of monoclonal antibodies specific for the PSA-ACT complex and their incorporation into an immunoassay. *Clin Chem* **43**:S225 (1997)

20 Christensson A, *et al.* Serum prostate specific antigen complexed to α-1-antichymotrypsin as an indicator of prostate cancer. *J Urol* **150**:100-105 (1993)

21 Catalona WJ, *et al.* Evaluation of free serum prostate -specific antigen to improve specificity of prostate cancer screening. *JAMA* **274**:1214-1220 (1995)

22 Luderer AA, *et al.* Measurement of the proportion of free to total prostate-specific antigen improves diagnostic performance of prostate-specific antigen in the diagnostic gray zone of total prostate-specific antigen. *Urol* **46**:187-194 (1995)

23 Henry JB: *Clinical Diagnosis and Management by Laboratory Methods, 18th Edition.* Henry JB ed, WB Saunders Company, Philadelphia, PA, pp 12-14, 78-79 (1991)

24 Stamey TA: Second Stanford Conference on International Standardization of Prostate-specific Antigen Immunoassays. September 1 and 2, 1994. *Urology* **45**(2):173-184 (1995)

25 Schroff RW, Foon KA, Beatty SM, Oldham RK, and Morgan AC Jr: Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy. *Can Res* **45**:879-885 (1985)

26 Boscato LM and Stuart MC: Heterophilic antibodies: A problem for all immunoassays. *Clin Chem* **34**(1):27-33 (1987)

27 Primus FJ, Kelley EA, Hansen HJ, and Goldenberg DM: "Sandwich"-type immunoassay of carcinoembryonic antigen in patients receiving murine monoclonal antibodies for diagnosis and therapy. *Clin Chem* **34**:261-264 (1988)

28 Koshe KP and Wisser H: Antibodies as a source of analytical errors. *J Clin Chem Biochem* **28**:881-892 (1990)

29 Courtenay-Luck NS, Epenetos AA, Winearls CG, and Ritter MA: Preexisting human anti-murine immunoglobulin reactivity due to polyclonal rheumatoid factors. *Can Res* 47:4520-4525 (1987)

30 Inloes R, Clark D, and Drobnies A: Interference of fluorescein, used in retinal angiography, with certain clinical laboratory tests. *Clin Chem* 33:2126-2127 (1987)

31 Morgan WR, Zincke H, Rainwater LM, Myers RP, and Klee GG: Prostate specific antigen values after radical retropubic prostatectomy for adenocarcinoma of the prostate: Impact of adjuvant treatment (hormonal and radiation). *J Urol* 145:319-323 (1991)